

ORIGINAL ARTICLE

Individual saturated fatty acids are associated with different components of insulin resistance and glucose metabolism: the GOCADAN study

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ABSTRACT

Objectives. Type 2 diabetes and the consumption of saturated fatty acids (FAs) are on the rise among Alaska Inuits. This analysis, based on a cross-sectional study, explores the possible associations of saturated FA content in red blood cells (RBCs) and parameters of glucose metabolism in a sample of Alaska Natives.

Study design and methods. The sample included 343 women and 282 men aged 35–74. Statistical analyses explored the associations of selected RBC (myristic, palmitic and stearic acids) FAs with fasting glucose (plasma), fasting insulin (plasma), 2h glucose (2-hour glucose tolerance test), 2h insulin and homeostasis model assessment (HOMA) index. The models included sex and glucose metabolism status as fixed factors and age, body mass index (BMI), waist circumference, physical activity (METS) and FA content in RBCs as covariates. Measures of insulin, glucose and HOMA index were used as dependent variables.

Results. Myristic acid was positively associated with fasting insulin ($\beta=0.47$, $p<0.001$), 2h insulin ($\beta=0.53$, $p=0.02$) and HOMA index ($\beta=0.455$, $p<0.001$). Palmitic acid was associated with 2h glucose ($\beta=2.3\times 10^{-2}$, $p<0.001$) and 2h insulin ($\beta=5.6\times 10^{-2}$, $p=0.002$) and stearic acid was associated with fasting glucose ($\beta=4.8\times 10^{-3}$, $p=0.006$).

Conclusions. These results strongly support the hypothesis that saturated fatty acids are associated with insulin resistance and glucose intolerance and that saturated fatty acids are significant risk factors for type 2 diabetes.

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Keywords: myristic acid, palmitic acid, stearic acid, Inuit, Alaska Natives, diabetes, saturated fat

INTRODUCTION

The dietary shift from highly unsaturated to more saturated fats currently being experienced by Alaska Natives presents an exceptional opportunity to study the effects of dietary differences on health. The population participating in the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study (1) is relatively homogeneous genetically and culturally, which makes it easier to identify associations between variation in consumption of specific FAs and alterations in phenotypic and pathological variables. Large studies ($n > 2,000$) of Alaska Natives 40 years ago clearly revealed a very low prevalence of type 2 diabetes (DM) ($< 0.4\%$) (2,3) and coronary heart disease (CHD) $< 2\%$ (4) when the dietary fat originated primarily from fish, whale, walrus and seal (5).

Recent studies of adult populations in western Alaska showed average DM prevalence ranging from 2.8 to 9.6% (6,7). It has been estimated that in 1975 the traditional fats (mostly monounsaturated and omega-3 fatty acids [FAs]) provided about 39% of the caloric intake, protein about 33% and carbohydrates about 28% (8). Now most foods are store-bought. Average caloric intake of carbohydrates has risen to 48% and the mix of fats consumed has changed to include, on average, twice the recommended caloric intake of saturated fats (9). The mix of fats now consumed range among individuals from nearly entirely traditional to completely store-bought, non-traditional fats (9,10). This variability allows for the investigation of associations of individual fatty acids consumed with emerging health problems. This "Alaska Native model" is now beginning to elucidate the role of specific FAs

in pathogenesis. Earlier studies have revealed a rapid increase in prevalence of DM (6), CHD (11) and cerebrovascular disease during the time of change in dietary FA consumption (8,11), but have also revealed important benefits of traditionally consumed fats (12,13).

The reasons for the increase of DM in Alaska Natives are not clear, although genetic predisposition and obesity are important risk factors in this population (6). Changes in specific dietary components also appear to increase the risk of developing DM in this population (10). The abandonment of the traditional high consumption of omega-3 fatty acids (FAs) and increasing saturated FA consumption, for example, appear to play a role in increasing glucose intolerance (10). Studies have suggested that omega-3 FAs may facilitate beta cell secretion of insulin and also increase insulin receptor activity (14) while excessive consumption of saturated FAs has the opposite effect (10,15,16). We have previously found associations between plasma levels of palmitate and impaired glucose tolerance (10) and between dietary consumption of palmitate and impaired glucose intolerance (13). The present report examines the association between the red blood cell (RBC) content of 3 individual saturated FAs with different components of insulin resistance and glucose metabolism.

MATERIAL AND METHODS

Study population

A total of 1,214 predominantly Inupiat (men and women) > 17 years of age from 9 villages in the Norton Sound Region of Alaska were examined between 2000 and 2004 for CVD and associated risk factors as part of the GOCADAN

study (1). In 7 of the 9 villages, an average of 82.6% of the eligible residents participated (17). From the sample of 1,214 participants, those between the ages of 34 and 75 (282 men and 343 women) were included in the present cross-sectional analysis to allow for a comparison with the same age group as in previous studies (6,13). Those with previously diagnosed DM and on medication for DM were excluded. Other reasons for restricting our analysis to this age group were (1) many of those older than 74 years of age were in poor health and on medication, and (2) the phenotypes of altered glucose metabolism are uncommon in those younger than 35 years of age.

Study examination

The GOCADAN exam (1) consisted of a personal interview (including medical history and medication use); physical examination including measurements of height, weight, waist circumference, percent body fat; ultrasound assessment of atherosclerosis in the carotid arteries; blood pressure (BP) measurements (following a 5-minute rest, sitting BP was measured on the right brachial artery 3 times with a Baum mercury sphygmomanometer [W.A. Baum, Copiague, NY]; the mean of the second and third measurements were used for analysis), ECG and a Rose questionnaire; blood sampling; and a nutritional interview using a validated food frequency questionnaire (FFQ) (9). Physical activity was measured with pedometers (steps/day). Readings over the course of 7 days were used to estimate physical activity (1). Highest and lowest values were eliminated and a trimmed mean was calculated for this variable. Participants were screened for type 2 diabetes by oral glucose tolerance test. Those with a new diagnosis of diabetes were

classified using the ADA 2003 criteria. HOMA index for insulin resistance was calculated by the following formula: Insulin (mU/ml) x glucose (mmol/l)/22.5 (18).

Laboratory analyses

A fasting blood sample was collected for measurement of glucose, insulin, lipids and lipoproteins (1). A (75 g) 2-hour glucose tolerance test was administered and blood was taken at 0 and 120 minutes for plasma measurements of glucose and insulin (1). HOMA index was estimated as described by Matthews et al. (18).

RBC fatty acid composition

RBCs were obtained from EDTA blood samples after removal of the plasma and buffy coat. A 1 ml aliquot was taken from the centre of the packed RBC pellet stored in -10°C for 1-4 days and then frozen in a 2 ml cryovial at -80°C. A 25 µl aliquot of thawed RBC pellet was transferred to a glass, screw-cap test tube, briefly vortexed and then heated at 100°C for 10 minutes with 250 µl of methanol containing 14% boron trifluoride. After cooling, 250 µl of hexane and 250 µl of water were added, and the tube was shaken for 30 seconds. After a brief centrifugation to separate layers, 50 µl of the hexane supernatant was transferred to a gas chromatography (GC) injection vial and capped. Samples were analysed on the day they were prepared or kept at -80°C until analysed. A GC2010 (Shimadzu Corporation, Columbia, MD) equipped with a 100 m capillary column (SP-2560, 0.25 mm internal diameter, 0.2 µm film thickness from Supelco, Bellefonte, PA) and a flame ionization detector were used. The run time was approximately 20 minutes using a temperature-programmed analysis (180°C held for 1.75 min, increased

by 5°C/min to 200°C and held for 1.75 min, increased to 240°C by 10°C/min, held for a final 7 min). Fatty acids were identified by comparison to a commercially prepared, weighed and standard mixture consisting of 22 FAs characteristic of RBCs (GLC-727, Nuchek Prep, Elysian, MN). The GLC-727 mix also served as an external standard for calculation of individual FA response factors. The FID response of palmitic acid was assumed to be 1.0, and the factors for all other FA were calculated and applied to the unknown samples on a run-by-run basis. Two RBC control pools, 1 high in omega-3 FAs and 1 low, were analysed with each run. The CV for very low abundance FAs (<1.0% of total FAs) was 22%; for low abundance FAs (1–10%), 7%; and for high abundance FAs (>10%), it was 5%.

Statistical analysis

Statistical analyses were conducted using SPSS v 9. The distribution of variables was tested using a Kolmogorov-Smirnov test. Variables with a non-normal distribution were log-transformed and tested again for normality before the selection of the statistical methods. We estimated and compared the genes of the analysed variables by sex using the Student's t-test. We conducted univariate analyses of both genders combined using a general linear model analysis, and only main effects were analysed for the proposed models. Fasting glucose levels, fasting insulin levels, HOMA index and values for glucose and insulin 2 hours after the intake of 75 g of glucose were analysed as dependent variables. The models were constructed using sex and glucose

metabolism category (normal or glucose intolerant according to ADA 2003 criteria) as fixed factors and log-transformed BMI, waist circumference and fatty acid content in red blood cells as covariates. Sex and glucose metabolism categories were binary variables. All covariates are parametric. Results of the analysed models are summarized in Table III.

RESULTS

The mean±SD age for participants was 48.5±9.9 years, with mean body mass index of 27.8±5.8 and percent body fat of 39.4±1.4% (Table I). No significant difference was found in age between men and women. Women had higher BMI than men, but similar waist circumference. Values for 2 h glucose and insulin were significantly higher among women in the study. Men had a higher intake of fat, but this amount represented about 35% of their caloric intake and was not different from women's percent fat intake. The RBC content of myristic acid, C14:0, palmitic acid, C16:0 and stearic acid, 18:0 did not differ significantly between men and women (Table II). Analyses revealed positive associations between myristic acid and fasting insulin ($\beta=0.47, p<0.001$), 2 h insulin ($\beta=0.53, p=0.02$) and HOMA index ($\beta=0.455, p<0.001$) (Table III). Palmitic acid was associated with 2 h glucose ($\beta=2.3\times 10^{-2}, p<0.001$) and 2 h insulin ($\beta=5.6\times 10^{-2}, p=0.002$), and stearic acid was associated with fasting glucose ($\beta=4.8\times 10^{-3}, p=0.006$). We failed to detect any significant association between total or individual trans fatty acids and these phenotypes.

Saturated fatty acids in RBC and glucose metabolism

Table I. Characteristics of study population: comparison of mean±SD of the analysed variables by sex.

	Male (n=282)	Female (n=343)	p
Age years	49.8±10.0	49.6±9.4	NS
BMI	26.4±4.7	28.5±6.2	<0.001
Waist circumference (in)	34.8±4.5	34.8±5.6	NS
Body fat (%)	33.0±5.4	44.5±4.8	<0.001
Fasting glucose (mg/dl)	93.3±8.9	92.9±8.4	NS
2 h glucose (mg/dl)	91.7±37.0	106.3±41.8	<0.001
Insulin (μU/dl)	8.8±2.0	9.8±6.3	NS
2 h insulin (μU/dl)	27.8±32.0	43.8±40.7	<0.001
HOMA index	2.1±2.0	2.3±1.7	NS
HbA1c	5.5±0.27	5.4±0.28	NS
Physical activity (steps/day)*	7,753.2±4,178	5,974.9±3,418	<0.001
Total fat intake (g/d)	178.6±140.8	133.7±104.6	<0.001
% fat from total calories	35.4±2.9	35.5±3.1	NS
TV hours (week)	19.6±16.7	13.9±11.6	<0.001

*These calculations excluded subjects with diabetes.

Note: p-value corresponds to Student's t-test.

Table II. Characteristics of study population: RBC phospholipid fatty acids (% of total fatty acids).

	Male n=282	Female n=343
Fatty acid		
SFA ^a		
14:0 ^b	0.35±0.14	0.42±0.17
16:0 ^c	20.60±1.7	21.10±1.9
18:0 ^d	14.23±13.56	13.97±13.65
Trans fatty acids ^e	2.09±0.58	1.95±0.5

^aSFA, saturated fatty acids

^bMA, myristic acid (14:0)

^cPA, palmitic acid (16:0)

^dSA, stearic acid (18:0)

^eTrans, total trans fatty acids: 16:1t+18:1t+18:2t

Table III. Beta coefficients and p values for variables in the univariate models.

variable	Fasting glucose		Fasting insulin		HOMA IR		2 hr glucose		2 hr insulin	
		p value		p value		p value		p value		p value
sex	0.21	0.75	0.41	0.45	0.1	0.03	0.16	<0.001	10.8	<0.001
GMS*	-14.95	<0.001	-2.9	<0.001	-0.4	<0.001	-0.09	0.02	-10.51	0.03
age	7.00E-002	0.02	4.40E-002	0.09	0.21	0.06	0.39	<0.001	0.46	<0.001
BMI	6.22	0.08	12.98	<0.001	0.99	<0.001	-0.12	0.53	54.1	0.86
waist	0.24	0.96	3.4	0.41	0.61	0.07	0.61	0.02	14.4	0.59
14 0 in RBC	1.92	0.27	0.47	<0.001	0.46	<0.001	0.03	0.25	0.53	0.02
16 0 in RBC	1.1	0.15	0.29	0.24	0.01	0.96	0.08	<0.001	5.60E-002	<0.01
18 0 in RBC	4.80E-003	<0.01	1.58	0.03	0.05	0.09	0.03	0.1	5.41	0.32

*Glucose metabolism status.

Note: Model includes saturated fatty acids in red blood cells in the table, age, log BMI and log waist circumference as covariates, and sex and glucose metabolism status (normal or glucose intolerant) as fix factors. Diabetic participants were excluded from the analyses.

DISCUSSION

This is the first study to show associations of individual RBC-saturated FAs with measures of human glucose metabolism in Alaska Natives. The findings show that the 3 saturated FAs play different roles than previously known and that the 3 FAs investigated here may affect such metabolism differently.

Some aspects of this specificity have been observed in other attempts at FA profiling, that is, when the whole spectrum of individual FAs was measured and individually associated with a particular phenotype or disease. The first such study among Alaska Native (n=454) related total plasma FAs to glucose metabolism (10). The study revealed a variety of associations between plasma levels of individual FAs and measures of glucose metabolism. Another study of a separate cohort of Alaska Native (n=633) documented similar associations using estimates of FA consumption from dietary recall data (13). Both of these studies showed a significant association between palmitate and 2 h glucose. The present RBC FA data strengthen the hypothesis that saturated FAs have adverse effects on glucose metabolism and suggest that high consumption, as reflected by RBC FA concentrations, of these FAs have predictable negative outcomes (10,15,16). The cross-sectional studies are also supported by a 4-year pilot intervention study to reduce consumption of saturated fats (19). This intervention study resulted in lower plasma concentrations of palmitate associated with improved glucose tolerance and the apparent prevention of DM.

The RBC FA values for palmitate in the present study provided a stronger association with 2 h glucose (p=0.001) than estimates of palmitate consumption by dietary recall in the

same cohort (p=0.07) (13). This may be because dietary data may have a larger measurement error. Taken together, all data from several studies on Alaska Natives show a significant association of palmitic acid with 2 h post-load glucose. Thus, the results of cross-sectional studies as well as the intervention study and a prospective study by Vessby et al. (15) support the hypothesis that high palmitate consumption contributes to the development of DM. The present cross-sectional study only shows possible impacts of various saturated FAs that need to be confirmed with longitudinal and randomized controlled studies.

The new data also indicate that other saturated FAs adversely affect various components of glucose metabolism as shown in a prospective study by Vessby et al. (15) and in an intervention study (16). In general, other studies have shown that saturated fats increase insulin resistance (15,16,20,21). Saturated fat consumption, especially palmitate (16) and myristic acid, impairs insulin sensitivity in healthy men and women (15,16,21,22). One large prospective study (n=24,155; 21) (22) involved individuals 40–79 years of age who attended a baseline health check and were later (mean 4.6 years) assessed for the development of DM on the basis of having achieved 5 intervention goals (BMI < 25 kg/m², fat intake < 30% of energy intake, consumption of saturated fats to < 10% of energy intake, fiber intake ≥ 15 g/4,284 kJ, physical activity > 4 h/week). The results showed that none of the participants who met all goals developed diabetes. It has been suggested that consumption of saturated fats should be < 7% of total energy intake (24). In the GOCADAN cohort, saturated fat consumption was 15% of the mean daily energy intake in the highest quartile of the population (9).

Proposed mechanisms

The differences in associations of the 3 FAs cannot be explained at this time and a specific hypothesis related to possible mechanisms to explain this finding does not exist. The biological processes linking saturated fats to insulin resistance are not resolved, but may relate to altered membrane phospholipid fatty acid composition and membrane fluidity and stability (24) and changes in lipogenic gene transcription (25). It is known that lipid metabolites that interfere with insulin signal transduction accumulate in tissues of insulin-resistant animals (6). In adult rat pancreatic islet cultures, a clear lipo-toxic effect of palmitic acid has been observed, which involved an increased apoptosis rate, coupled with a reduced proliferation capacity of beta cells and impaired insulin secretion. The deleterious effect of palmitate on beta cell turnover is mediated by the formation of ceramide and the activation of the apoptotic mitochondrial pathway (26). Since our original study involving Alaska Natives (10), studies on rats have shown that high levels of palmitate lead to insulin resistance due to changes in the level of phosphorylation of the insulin receptor and insulin receptor substrate (IRS)-1 (24).

The current analyses reveals significant FA specific associations with phenotypic variables related to glucose metabolism not contemplated only a few years ago. The specific metabolic mechanisms are not currently known, but likely involve gene-specific FA interactions.

Limitations and strengths

The present cohort principally represents the Inupiat peoples; so these data may not necessarily be representative of other ethnic groups. Although the participation rate was exceptionally high (83% in 7 villages), the study popula-

tion may not fully represent the population, as some of unknown age and gender did not participate because of temporary absence from a given village. Also, since the present study is limited to the association of phospholipid bound FAs in RBC membranes to insulin resistance and glucose metabolism, future research on other measures of non-phospholipid FAs in relation to glucose metabolism are warranted by the present findings. The strength of the study is the clear results due to the exceptional genetic and cultural homogeneity of this cohort presently undergoing an acculturation with a broad exposure to different fats. This allows the study of individual FAs in relation to health status and related phenotypes.

Conclusions

Individual saturated FAs are adversely associated with different components of insulin resistance and glucose metabolism. The cumulative evidence from controlled dietary studies (16,21), cross-sectional studies (10), prospective studies (15) and intervention studies (16,19,21) agree that saturated FAs have detrimental effects on insulin resistance and glucose metabolism (26). The current shift towards higher consumption of saturated fats among Alaska Natives is clearly detrimental to their health. These results justify the initiation of ethnic-specific intervention and prevention studies to reduce saturated fat consumption.

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